Scientific Abstract

The use of multiagent chemotherapy combined with surgery and radiation therapy has permitted significant advances in the treatment of many solid tumors, particularly those occurring in childhood. Many sarcomas in both adults and children, however, are relatively resistant to chemotherapy and radiation therapy, leaving surgery as the only treatment option. With current high dose intensity therapies, fewer than half of patients with metastatic neuroblastoma are alive at 5 years from diagnosis, and fewer than 30% of patients with metastatic sarcomas are alive at 5 years. Therefore, new treatment options are desperately needed for these diseases.

Attenuated Herpes simplex virus (HSV) mutants engineered with deletions of normally critical genetic functions dispensable in cancer cells are being actively pursued as novel therapeutic agents. rRp450 is derived from the HSV-1 strain KOS, but is deleted for the ribonucleotide reductase gene, causing it to be permissive for replication only in rapidly dividing cells. It expresses the cloned rat CYP2B1 gene, a prodrug converting enzyme. In preclinical studies, rRp450 not only kills tumor cells by direct lysis, but also sensitizes them to prodrugs such as cyclophosphamide. Using cell lines and models of subcutaneous human xenograft tumors in mice, we have demonstrated a significant antitumor effect following injections of rRp450 alone (in the absence of prodrugs) for a variety of sarcomas and neuroblastomas. Therefore, we propose a phase I clinical trial to test the safety of direct intratumoral injections of rRp450 in patients with high-risk sarcomas and neuroblastomas.

Patients will be eligible if they have relapsed or refractory disease. Metastatic patients at relapse will be included, in which case a single lesion will be chosen for administration. Because of concerns regarding administering virus to children, at each dosage level a cohort of adult patients (22-30 years old) will be enrolled first, followed by a cohort of children. Once that dose level has been shown to be safe in these adult patients, three children (1-21 years old) will be enrolled at the same dose level (infants <1 year old will be excluded). A total of six dose strata will be planned, with a maximum pre-determined by the feasibility of virus concentration. The virus will be administered directly into the tumor via CT-guided injection by interventional radiology. Because the virus will be administered locally and not systemically, the dose will not be adjusted for patient size. Patients will be able to receive a total of four injections at least 3 weeks apart to determine the safety of single as well as multiple administrations.

The dose to be used will be limited by reasonable volumes for intratumoral injections. In our experience with xenograft tumors in mice, no more than about 100 μl can be placed at a given injection site without significant loss through the needle tract. Allowing for up to 5 injections in a given tumor, the maximum volume to be injected will be 500 μl. Given viral stock concentrations that are typically in the range of 10^{10} pfu/ml, our maximum dose will be 5×10^{9} pfu. The adult dose shown to be safe so far with intra-arterial infusion of NV 1020 is 1.3×10^{7} pfu, though the maximum tolerated dose (MTD) has not yet been reached and the dose-escalation is ongoing. It is typical practice in pediatric phase I trials to begin with a dose that is 80% the adult MTD. Although no MTD is yet known, our first dose stratum will be 80% of the adult dose already shown to be safe. We

will therefore begin at a dose of 1 x 10^7 and dose-escalate in half-log increments to the maximum dose (6 dose strata). Therefore, the total study will require 6.66 x 10^9 pfu for 1 patient at each stratum x 6 patients (3 adults followed by 3 children at each stratum), for a total of 4 x 10^{10} pfu. The protocol will allow for a total of 4 injections at 3-week intervals, so the maximum amount of virus to be used in the trial is 1.6 x 10^{11} pfu.

As part of the trial, we will include biology studies including pre- and post-therapy measurements of anti-HSV1 immunity, serum cytokine expression, and quantitative real-time polymerase chain reaction measurements of viremia (using primers and probes specific for the rat CYP2B1 gene to distinguish the therapeutic reagent from natural HSV I infection). Patients will be monitored long-term for evidence of viral latency. In cases where a post-therapy tumor biopsy is clinically indicated, the extent of viral replication by in situ hybridization and correlation with tumor cell necrosis will be evaluated.

This study will establish a safe dose for intratumoral administration and thus facilitate the design and implementation of subsequent clinical trials of rRp450 for sarcomas and neuroblastomas.